PERIODICITY IN SPIROGYRA, WITH SPECIAL REFERENCE TO THE WORK OF BENECKE.*

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The fact that there is a certain periodicity in the appearance of reproductive phases of many algae is a matter of common observation, and several attempts have been made to determine more or less definitely the factors controlling reproduction in these forms. In work of this kind *Spirogyra* has perhaps been the most frequent subject for experimentation and observation, but the work of Williams ('05) and Hoyt ('07) on *Dictyota* has yielded interesting, if somewhat puzzling results. The former of these authors was able to demonstrate a very constant and clearly marked periodicity in *Dictyota dichotoma* which he found to liberate the eggs and sperm at a definite time following the highest spring tide of each cycle. An analysis of the conditions that vary concomitantly with the tide, and the fluctuations in the tide and time of sexual maturity of *Dictyota* on the various parts of the British coast led the author to believe that the observed periodicity in the reproduction of this plant is controlled by the amount of light received, which in turn is regulated by the tides. Nevertheless plants kept in an aquarium were found to show the same periodicity the following spring despite the fact that they had been for a long time removed from all periodic fluctuations except those of day and night. Williams therefore concluded that the periodicity manifested by *Dictyota dichotoma* is an inherited characteristic which in nature comes to synchronize with the tidal periods.

Hoyt studied the same species on this side of the Atlantic

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and while his data as to the time of liberating the sexual products do not exactly coincide with those of Williams, the general features of the cycle are the same and he too was able to demonstrate a periodicity in aquarium plants that agreed closely with that shown under natural conditions, even though the gametangia were born on parts that had developed entirely in the laboratory and had consequently never been subjected to the usual periodic variations in the environment. He also obtained some evidence to the effect that plants growing in seas where there are almost no tides likewise show a similar periodicity but, as the author remarks, the evidence on this point is perhaps not sufficient. In short the results of both these investigators indicate that in *Dictyota dichotoma* a periodicity in the production and liberation of the sexual products, while seemingly influenced to a marked degree by environmental fluctuations, is primarily due to an inherent periodic tendency on the part of the plant itself.

Whether *Spirogyra* is similar to *Dictyota* in respect to its periodicity has not been definitely settled. Fritsch and Rich ('07) made rather extensive field observations on a number of species of *Spirogyra* and came to the conclusion that the stimulus to conjugation is probably an external one consisting of very complex factors and that it is presumably different for the different species. These observers point out that the same species in the same body of water conjugates at varying times in different seasons and that different species under similar conditions do not behave in like manner during the same season. Brown ('08) likewise made numerous observations on *Spirogyra* and other forms in the field and was led to conclude that these algae grow on indefinitely so long as conditions in the environment do not become adverse. *Spirogyra* conjugates when conditions become "hard enough." Copeland ('09), on the other hand, kept a large number of cultures in aquaria and supplemented a study of them with observations on *Spirogyra* in nature. His results indicate that *Spirogyra* has definite periods of growth and activity and he finds "overwhelming evidence....that

....conjugation results not so much from external as from internal conditions."

Of a somewhat different nature is the work of Benecke ('08) which is entirely experimental in character. This investigator put *Spirogyra communis* in different media (water or salt solutions) in aquaria of various sizes. They were for the most part placed in bright light and kept at a temperature ranging from 12 degrees to 20 degrees C. He found, as his tabulated data show, that in nitrogen-free solutions conjugation took place in a short time. The number of zygotes formed, however, varied somewhat in the different media, distilled water being one of the least favorable. If parallel cultures were run in which $-NH_4$ or $-NO_3$ had been added in appropriate amounts (about .05%) to any of the above media or substituted for one of the constituent salts, no conjugation took place at all but generally a good vegetative growth ensued. As a result of his work Benecke believes that conjugation in *Spirogyra* is due to the failure of ammonium salts which he supposes to be removed from the water by angiosperms which increase in size and abundance as the season advances.

Benecke's work seeming to be of a very definite character, an attempt was made by the writer during the winter of 1909-1910 to repeat these experiments using other species in the place of *S. communis.* The result has been almost a complete lack of conformity in so far as the question of zygospore formation is concerned. The stimulating effect of an ammonium salt on growth, however, frequently proved to be quite as marked as it was with *S. communis* in Benecke's cultures. The species used for the present study were chiefly *S. setiformis, S. longata, S. Grevilleana, S. dubia,* and *S. porticalis.* There was also a large and very resistant form which occurred for the most part as scattered filaments associated with the other species and generally persisting in the aquaria after the associates had died. Unfortunately *S. communis* was not accessible.

In the fall, *S. setiformis* was the most available form. It was found in considerable abundance in one of the small,

shallow, "tropical ponds" at the Garden and remained there till the pond was covered for the winter. Good vegetative material and occasional conjugating filaments could be found throughout the fall but the species did not reappear in the spring, due possibly to interference of the workmen. December 10, some of this material was brought into the laboratory and placed in media made up according to Benecke's formulae for his second series. The solutions are as follows:

No. 1. Distilled water.

No. 2. Tap water (In place of pond water.)

No. 3. Distilled water, 100 cc.

KNO_3,	.05 grm.
$Ca_3P_2O_8$,	.05 "
$Fe_3P_2O_8$,	.05 "
$MgSO_4+7H_2O$,	.05 "

No. 4. Like No. 3, but in place of KNO_3, KCl, .04 grm.

No. 5. Distilled water, 100 cc.

KNO_3,	.05 grm.
$CaCl_2$,	.05 "
$FeSO_4+7H_2O$,	.005 "
$MgSO_4+7H_2O$,	.05 "

It will be observed that Nos. 3 and 5 alone contain NO_3 in solution. When Benecke experimented with *S. communis* in these media he found that in the course of eleven days Nos. 1, 2, and 4 produced zygotes and No. 5 showed abnormal developments while in No. 3 no zygotes were formed but a good vegetative growth took place. In my cultures Nos. 1, 2, and 5 had died by the end of the third week without having conjugated, but most of the filaments in Nos. 3 and 4 were in good condition and had apparently grown slightly. Filaments in these two cultures remained alive in the laboratory under conditions that seemed favorable for conjugation from December 10 till after the first of April when most of them gradually died, no conjugation having taken place in either solution although from Benecke's results it was to have been expected in No. 4.

Solutions No. 3 and No. 4 of Benecke's third series were also used at this time. Their composition is:

No. 3. Tap water, 1500 cc.

NH_4NO_3,	.01%
$CaCl_2$,	.005%
K_2HPO_4,	.005%
$MgSO_4+7H_2O$	.005%
Fe_2Cl_6	1 drop of the standard solution.

No. 4. The same as No. 3 except for the omission of the NH_4NO_3.

The exact formulae given were departed from to the extent of substituting distilled water for tap water, the composition of the latter being unknown. With Benecke No. 3 gave good vegetative growth and No. 4 numerous zygotes, but in this case, although many of the filaments in both cultures lived and apparently grew to a slight extent, no conjugation took place during the five weeks in which the solutions remained unchanged. January 14, some melted snow was added to these cultures. The filaments in solution No. 4 died during February, those in No. 3 persisted until April. There was no conjugation in either. In regard to the several cultures started December 10 it is of interest to note that those in tap and distilled water died, as did also several cultures in which NH_4NO_3 alone was added to the water, while the more elaborate media proved favorable. No. 5 of the second series, here as elsewhere, appeared to be slightly toxic, probably due to the $FeSO_4$ contained. It may be remarked at this point that *S. setiformis* has not seemed to respond to NH_4NO_3 by vegetative growth as have *S. dubia* and *S. longata.* It died in the above mentioned solutions and when a slight trace was added to a culture of five months' standing in No. 4 of the second series, the filaments which previously had been in perfect condition became gnarled and distorted.

One other series in which *S. setiformis* was used may be mentioned. Late in February or early in March zygospores began to germinate in an aquarium where conjugating material had been left in the fall and by the first of April the majority of the filaments were from 15 mm. to 20 mm. in length. All the different salt solutions which Benecke had found to favor or permit conjugation in *S. communis* were made up according to his formulae except that in place of the occasional pond or tap water 100 cc. of twice distilled water that had been further treated with iron hydrate was used in each case. April 5 a few filaments were placed in each of these media and kept in the comparatively uniform temperature and good illumination of the laboratory. April 19, when filaments of the original stock had about doubled in length, only one or two of these cultures showed any growth

at all and in these cases it was very slight. Nevertheless no conjugation had taken place and there was none observed subsequently. Despite the precautions, mycelium developed in these cultures and soon affected the algae.

Spirogyra longata was the most abundant form throughout the greater part of the period during which the work was being carried on and served for a large number of experiments. During January, February, and the early part of March it seemed to be confined to a region near the source of a small stream that arises from an artificial pond in the Garden. Late in March it spread into the pond and down the brook and in April and May became excessively abundant. The environmental conditions certainly varied greatly during this period but only a very little conjugating material could be found, although in the same brook *S. Grevilleana* and possibly also *S. porticalis,* appeared, conjugated, and for the most part disappeared. Despite its apparent hardiness under natural conditions *S. longata* proved rather a difficult species to handle in the laboratory. When brought indoors, especially during the winter, it almost invariably grew rapidly for a few days, then suddenly fragmented and died. It acted in the same manner in a number of media, in bright or subdued light, and under somewhat varied conditions of temperature. Later in the season this tendency was much less pronounced. It was found, however, that even during the winter if flasks containing the cultures were placed at once in the brook the plants did not die and could subsequently be returned to the laboratory where they would then live for a long time. Later it was noticed that the addition of a slight amount of NH_4NO_3 a day or two after the plants were first brought to the laboratory served to prolong their life and stimulate growth, but of course this method could not be used with material intended for experimentation. Attempts to induce conjugation were without success except where the stock material had already shown at least incipient stages before the work was begun. Seventeen of the Benecke solutions were made up in melted snow, *S. longata* was added, and the aquaria (flasks) were kept in the stream from March

29 to April 12 and then in the laboratory for a longer period. Early in the course of this experiment the filaments in solution No. 5 of the second series began to twine around each other and even to entwine themselves, and those of No. 1 of the eleventh series (.005% each of Na_2HPO_4 and NaH_2PO_4) developed very marked false branches which did not resemble conjugation tubes but were much larger and longer. Several of the other cultures showed distortions of a less marked character but in none was there any conjugation whatever. The abnormalities referred to above have appeared from time to time in very different solutions but sufficient data are not at hand to warrant any opinion as to whether they are due to osmotic or toxic factors or represent an incomplete response to some stimulus to conjugation. A number of similar experiments with this form need not be considered beyond noting that Benecke's methods when tried under various conditions uniformly failed to produce conjugation except in the single case to be described in the following paragraph.

April 29, a mass of *S. longata* was found conjugating. Filaments that had already conjugated were pulled out from the rest and the remaining material (A) was used to start four cultures, two in No. 3 of Benecke's second series and two in No. 4. One of each was placed in the laboratory, the others in the brook. Four parallel cultures (B) were identical except that the material was from another mass where no indications of conjugation were to be found. May 10 the material from lot A which was placed in the pond showed vigorous growth in No. 3 and more or less conjugation (but no zygotes) in No. 4. These results approximate those of Benecke, the only clear case so far as this species is concerned. The similar cultures left in the laboratory showed stationary conditions in No. 3 and for the most part death in No. 4. The cultures in lot B showed good growth in the field and an indifferent condition in the laboratory. Although No. 4 in the field at first showed a very few tube-like outpushings these did not develop and no conjugation took place. The eight cultures were then put in the bright light of a south window

and re-examined on May 30 when it was found that all the material from the original lot A was either dead or greatly reduced while the four cultures from lot B which had received identical treatment were in good vegetative condition although there were individual differences between them.

Three other cultures started on April 29 consisted of conjugating material placed in aquaria of 500 cc., each kept in bright light but not in much direct sunshine. No. 1 was pond water which had been strained for the partial removal of animalculae. No. 2 was the same plus .008% each of Na_2HPO_4 and K_2HPO_4. In No. 3 these salts were replaced by NH_4NO_3, .016%. On May 6 the filaments in No. 1 were prostrate but the few zygotes appeared to be normally developed. No. 2 showed excellent vegetative growth in addition to the zygospores. The filaments in No. 3 were growing vegetatively and some of the zygospores appeared normal but many were bright green with a clear space either at one end or in the middle. In these clear spaces rapid Brownian motion such as is characteristic of the vegetative cells could be observed. May 10 apparently no additional zygospores were forming and the aquaria were placed in bright sunlight. By May 30 the filaments in No. 1 were entirely gone, those that were alive in No. 2 were in good condition and apparently taking a new start, and those in No. 3 were at a low ebb, the stimulating effect of the NH_4NO_3 having passed. None of the zygospores had germinated up to this time.

Small amounts of *S. Grevilleana* and *S. porticalis* were available during a part of March and early April. March 18 three sets of solutions were prepared; distilled water and Nos. 3 and 4 of Benecke's second series. These were used as stock solutions and from each of them were started two cultures of *S. longata,* two of *S. Grevilleana* and two of *S. porticalis. S. longata* had not been found fruiting, but both of the other species were conjugating at the time in nature. These cultures were examined on March 22 and it was found that *S. longata* and *S. porticalis* were vegetative throughout with no evidences of conjugation. *S. Grevilleana* likewise was vegetative in distilled water and in both solutions from No. 3, although

in one of these there were slight indications of tubes. But in both cultures of the No. 4 solution there was evident conjugation. Thus one of the three species partially fulfilled the expectations of Benecke's theory, while the other two failed entirely to do so. A larger series gave conjugation in eleven cases out of an expectation of fifteen with *S. Grevilleana,* but no slightest indication of it in parallel cultures of *longata* which were treated in identically the same manner. As was stated above, *S. Grevilleana* was conjugating in nature at the time the observations were made, and consequently it cannot be predicted how it would act during a period when conjugation is not normally occurring. Later in the season, however, when this species had become scarce occasional filaments sometimes occurred in my cultures associated with other forms; at this time, however, they did not conjugate, as would have been expected, but grew vegetatively.

On several occasions masses of actively conjugating *S. dubia* were brought into the laboratory and placed in cultural solutions. After several days a few filaments presenting the normal vegetative appearance could generally be found in all the solutions. The origin of these filaments was not determined with certainty, and while they seemed to be derived from filaments that had in part conjugated, it is not entirely impossible that they were younger plants that would have remained vegetative even in nature. Their subsequent fate in the laboratory varied, but those in the more complex salt solutions generally showed the most vigorous growth, No. 4 of the second series (which Benecke found to favor zygospore formation) being about the most favorable.

The germination of the zygospores in this species is of interest in view of the fact that in some cases it occurred after a very brief time. Zygospores that were forming about April 15 and at that time placed in solution No. 4 of the second series were germinating abundantly by May 3 when some of them had already become three cells long. They were also actively germinating after the same length of time in No. 4 of series ten (.02g. $NaNO_3$ in 150 cc. of water) and to a much less extent in No. 3 of series two. In pond and tap

water, the other two media used in this experiment, young plants could not be found at this time, although by May 30 many of those in the tap water had also germinated. In like manner when conjugating material of the same species, but from a different locality, was placed in solutions Nos. 3 and 4 of Benecke's second series and also in tap water, distilled water, and a solution containing .008% NH_4NO_3 there was, at the end of four weeks, more or less germination of zygospores in each case, although to a less extent in the tap water and distilled water. With the other species a similar germination did not seem to occur. Whether or not *S. dubia* germinated in nature at this time could not be satisfactorily determined, for while no filaments were seen it may have been because they were eaten by the large number of tadpoles that appeared in the ponds.

Briefly to summarize the results obtained, it appears that there are specific differences as regards the reactions of filaments and zygospores in the species studied, and that Benecke's conclusions, based on the reactions of *S. communis,* are probably not of general application, or are applicable only under very special conditions. Of the five species investigated three failed entirely to give the expected results, and a fourth failed in every case but one. The remaining species, *S. Grevilleana,* seems to agree more closely with *S. communis* but even here the agreement is not complete. The existence of sexual strains, such as occur in some of the moulds, seems to be suggested, but evidence on this point is lacking.

When Benecke's results are analyzed it becomes apparent that he did not find any specific stimulus that would induce conjugation unless the absence of ammonium salts be taken as such. The foregoing observation seems to show very clearly that in many cases at least the absence of these salts is not enough to bring about conjugation. Hence it seems all the more probable that, as Fritsch and Rich have stated, the conditions governing conjugation in this genus are very complicated and probably not always of such simple nature as Benecke is inclined to believe. Indeed it is still possible that *Spirogyra* like *Dictyota* is inherently periodic in its func-

tions, although its periodicity may be extensively influenced by the environment.

Literature Cited.

Benecke, W. Über die Ursachen der Periodicität im Auftreten der Algen, auf Grund von Versuchen über die Bedingungen der Zygotenbildung bei *Spirogyra communis*. (Internat. Rev. d. Gesamt. Hydrobiologie u. Hydrographie. **1**: 533–552). 1908.

Brown, Harry G. Algal periodicity in certain ponds and streams. (Bull. Torr. Bot. Club. **35**: 223–248). 1908.

Copeland, W. F. Periodicity in *Spirogyra*. (Bot. Gaz. **47**: 9–25). 1909.

Fritsch, F. E. and Florence Rich. Studies on the occurrence and reproduction of British freshwater algae in nature. I. Preliminary observations on *Spirogyra*. (Ann. of Bot. **21**: 423–436). 1907.

Hoyt, W. D. Periodicity in production of sexual cells in *Dictyota dichotoma*. (Bot. Gaz. **43**: 383–92). 1907.

Williams, J. Lloyd. Studies in the Dictyotaceae. III. The periodicity of the sexual cells in *Dictyota dichotoma*. (Ann. of Bot. **19**: 531–560). 1905.